PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference JWJ01058WO	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/GB2004/003086	International filing date *(day/month/year)* 15.07.2004	Priority date *(day/month/year)* 15.07.2003
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant DENSHAM, Daniel Henry		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15.02.2005	19.07.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Leber, T Telephone No. +49 89 2399-7195

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/003086

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-16 as originally filed

Claims, Numbers

1-18 received on 09.05.2005 with letter of 06.05.2005

Drawings, Sheets

1/2-2/2 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☒ the claims, Nos. 1(partly); claims 6, 10 (fully)
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/003086

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3,7,8,11-15,17,18
	No:	Claims	1,2,4,5,9,16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-5,7-9,11-18
Industrial applicability (IA)	Yes:	Claims	1-5,7-9,11-18
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item I

Basis of the opinion

1. Claim 1 has been amended to refer to a multiplex reaction. Part (ii) of claim 1 defines the methods of detection. The first option refers to "the molecule being located in a spatially defined position" and the second option to the molecule "being determined via a non-linear or non-fluorescent technique".
The application as originally filed, however, appears to disclose a multiplex reaction only in the context of the first detection option (see page 14, third paragraph), but not to the second. Amended claim 1 thus fails in part to comply with Art 34(2)(b) PCT.

2. Claim 6 refers to a molecule which "does not act as a primer". The application as originally filed, however, does not provide a basis for this amendment. The first paragraph on page 6 of the description, for example, only states that "this sequence may or may not be one which takes part in the amplification reaction". This, however, is not an unambiguous disclosure of the sequence being a primer or not as other oligonucleotides, such as labelled detection oligonucleotides or blocking oligonucleotides may also be used in an amplification reaction. Thus, claim 6 contravenes Art 34(2)(b) PCT.

3. Claim 10 refers to metallic particles. The description, however, only refers to gold particles (page 8, lines 9-11).Thus, claim 10 contravenes Art 34(2)(b) PCT.

In view of the above objections under Art 34(2)(b) PCT, the present IPER was established as if the unallowable amendments had not been made (Rule 70(2)(c) PCT). The IPER is based on claim 1 to the extent it is allowable under Art 34(2)(b), claims 2-5,7-9 and 11-18. No examination is carried out for claim 1 in part (see above), claims 6 and 10.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Basis for the assessment of novelty, inventive step and industrial applicability

1.1 Reference is made to the following document/s/:

D3: WO 96/09407 A (PHARMACIA BIOSENSOR AB ; NILSSON PETER (SE); NYGREN PER AAKE (SE); UHL) 28 March 1996 (1996-03-28)

D4: KAI E ET AL: "DETECTION OF PCR PRODUCTS IN SOLUTION USING SURFACE PLASMON RESONANCE" ANALYTICAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. COLUMBUS, US, vol. 71, no. 4, 15 February 1999 (1999-02-15), pages 796-800, XP009004116 ISSN: 0003-2700

D5: FERIOTTO GIORDANA ET AL: "Quantitation of bt-176 maize genomic sequences by surface plasmon resonance-based biospecific interaction analysis of multiplex polymerase chain reaction (PCR)." JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY. 30 JUL 2003, vol. 51, no. 16, 27 June 2003 (2003-06-27), pages 4640-4646, XP002301029 ISSN: 0021-8561

D6: BIANCHI N ET AL: "Biosensor technology and surface plasmon resonance for real-time detection of HIV-1 genomic sequences amplified by polymerase chain reaction." CLINICAL AND DIAGNOSTIC VIROLOGY. NOV 1997, vol. 8, no. 3, November 1997 (1997-11), pages 199-208, XP002301030 ISSN: 0928-0197

1.2 The amendments filed with the letter of 06.05.2005 do not fulfill the requirements of Art 34(2)(b) PCT (see Item I, above)

2. Novelty

2.1 D3 discloses a process for the quantification of a target nucleic acid in a sample comprising the steps of: (i) adding to the sample containing said target nucleic acid a known amount of a competitor nucleic acid; (ii) amplifying both the competitor and target nucleic acids in parallel by PCR (=multiplex); (iii) immobilizing the amplified nucleic acids onto a biosensor sensing surface; and (iv) subjecting the respective immobilized nucleic acids to a biospecific interaction or interactions, and from the changes in a property of the sensing surface caused by the interactions of the respective nucleic acids determining the relative amounts of the target and competitor

nucleic acids to thereby determine the amount of said target nucleic acid in said sample (see abstract). In a specific embodiment the target DNA or RNA fragments are captured/immobilized via immobilized wildtype or competitor specific probes and the sequence specific capture is followed by the detection of enzymatic extension (see page 6, l. 32-37). Biosensor-based detection means are used to monitor, preferably in real-time, the enzymatic manipulation of the immobilized nucleic acid fragments (see page 4, l. 14-22). A preferred detection method uses SPR (see page 5, l. 1-6).
Thus, in view of D3 the subject matter of claims 1,2,4,5,9,16 is not novel (Art 33(2) PCT).

2.4 D4 discloses the detection of PCR products in solution using surface plasmon resonance (SPR). Asymmetric PCR using several sets of primers (=multiplex PCR) was used to amplify the target DNA sequence, and two products with different length were produced; the so produced target DNA was double stranded but the probe binding site, located in the 3-terminus, was single stranded (see abstract). The PCR products were detected by SPR using a probe immobilized on the surface of a sensor chip which was complementary to the single stranded region of the asymmetric PCR product (see Fig. 1, page 797). The detection system is capable of detecting PCR products quantitatively (see page 800, last paragraph).
Thus, in view of D4 the subject matter of claims 1,2,4,9,16 is not novel (Art 33(2) PCT).

2.5 D5 discloses a method for the Quantitation of bt-176 maize genomic sequences by surface plasmon resonance (SPR)-based biospecific interaction analysis (BIA) of multiplex polymerase chain reaction (PCR). The design and testing of an SPR-based BIA protocol for quantitative determinations of PCR products is described. Biotinylated multiplex PCR products were immobilized on different flow cells of a sensor chip. After immobilization, different oligonucleotide probes recognizing maize zein and Bt-176 sequences were injected. The efficiency of SPR-based BIA in discriminating material containing different amounts of St-i 76 maize is comparable to real-time quantitative PCR (see abstract; Fig. 2; page 4644, "Discussion").
Thus, in view of D5 the subject matter of claims 1,9,16 is not novel (Art 33(2) PCT).

2.6 D6 discloses the determination of the specific hybridization of a biotinylated HIV-1 oligonucleotide probe immobilized on a sensor chip to single stranded DNA obtained by asymmetric polymerase-chain reaction (PCR) using the BIAcore biosensor whereby different products are simultaneously are amplified (Fig. 1A). Direct injection of asymmetric PCR to a sensor chip carrying an internal HIV-1 oligonucleotide probe allowed detection of hybridization by SPR using biosensor technology (see abstract). Thus, in view of D6 the subject matter of claims 1,2,9,16 is not novel (Art 33(2) PCT).

3. Inventive step (Art. 33(3) PCT)

3.1 The subject matter of claims 8 and 9 relates to a method for monitoring the production of multiplexed PCR products wherein the interaction of the polymerase enzyme with the amplified product is detected real-time by measuring changes in applied radiation induced upon interaction between the reaction products and immobilised molecules. The description of the present application does however not disclose any data demonstrating the achievement of the intended technical effect of such method. The ISA can therefore not acknowledge an inventive step for the subject matter of claims 8 and 9; the reasons are the following:
Due to the fact that the patent specification does not demonstrate the achievement of the desired technical effect of the subject matter claimed in claim 8 and 9, the subject matter of claim 8 and 9 cannot be considered to represent a solution to a technical problem. Inventive step must therefore be denied (Art 33(3) PCT).

3.2 Dependent claims 3, 7, 11-15,17 and 18 refer to subject-matter which in combination with the subject-matter of any of the claims to which they refer appears not to meet the requirements for inventive step (Art 33(3) PCT) as the features to which they refer fall within the range of modifictions routinely applied by the skilled person working with (multiplex)-PCR and the detection of the thereby generated products (Art 33(3) PCT).

4. Industrial applicability

4.1 The subject-matter disclosed in the claims 1-5,7-9,11-18 of the present application appears to be industrially applicable (Art 33(4) PCT).

CLAIMS

1. A method for monitoring the amplification of a plurality of different target polynucleotides in a single reaction chamber comprising the steps of:

(i) carrying out a reaction for the amplification of a plurality of different target polynucleotides;

(ii) during the amplification reaction contacting different amplified products with a molecule that binds to or interacts with a polynucleotide, the molecule being located in a spatially defined position or being determined via a non-linear or non-fluorescent technique; and

(iii) detecting the interaction between the amplified product and the molecule by measuring changes in applied radiation.

2. A method according to claim 1, wherein the molecule is immobilised to a support material.

3. A method according to claim 1 or claim 2, wherein the molecule is a polymerase enzyme.

4. A method according to claim 1 or claim 2, wherein the molecule is a polynucleotide, at least a portion of which is complementary to a region on an amplified product.

5. A method according to claim 4, wherein the molecule acts as a primer for the amplification reaction.

6. A method according to claim 4, wherein the molecule does not act as a primer for the amplification reaction.

7. A method according to any preceding claim, wherein deletion in step (iii) is carried out by detection of an evanescent field.

8. A method according to any preceding claim, wherein detection in step (iii) is carried out by applying surface electromagnetic waves and monitoring changes in the waves.

9. A method according to claim 7 or claim 8, wherein detection is carried out by measuring changes in surface plasmon resonance.

10. A method according to claim 9, wherein the molecule comprises a metallic particle.

11. A method according to any of claims 1-6, wherein detection in step (ii) is carried out by detecting surface enhanced Raman scattering.

12. A method according to any of claims 4-6, wherein detection in step (ii) is achieved by detecting an intercalating label that binds to the hybrid formed between the amplified product and polynucleotide during the amplification reaction.

13. A method according to claim 12, wherein the intercalating label is fluorescent when bound to the hybrid.

14. A method according to any of claims 1-6, wherein detection in step (iii) is achieved by monitoring changes in electrical conductance and/or capacitance.

15. A method according to any preceding claim, wherein the amplification reaction occurs in a sealed micro-flow cell.

16. Apparatus for monitoring a polynucleotide amplification reaction, comprising a support material having a plurality of molecules immobilised

thereon, the molecules having the ability to bind to or interact with a polynucleotide, and means for detecting changes in applied radiation.

17. An apparatus according to claim 16, further comprising a sealed micro-flow cell.

18. An apparatus according to claim 16 or claim 17, further comprising a pump to maintain a flow of fluid over the support material.